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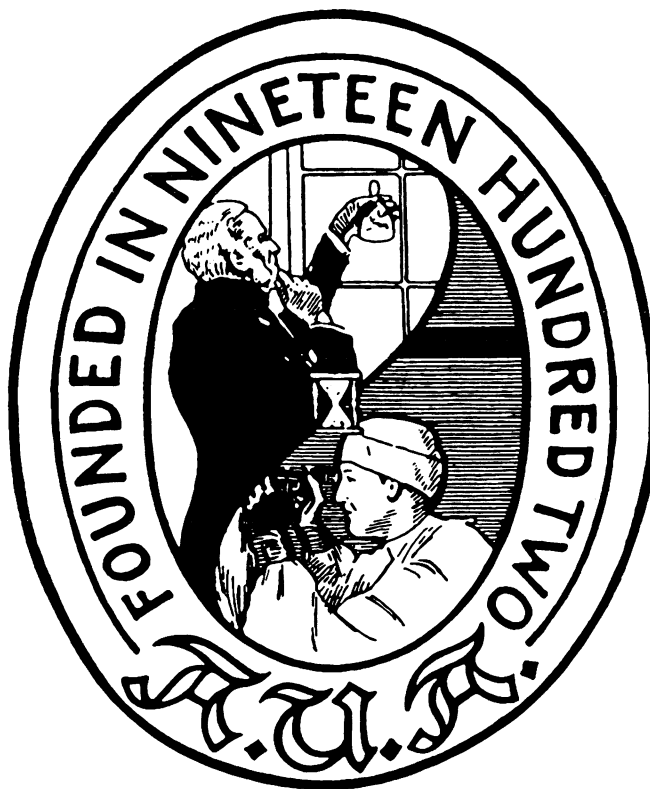
Volume 142

July 1989

Number 1

The Journal of UROLOGY®

Official Journal of the American Urological Association, Inc.
Founded In 1917 By Hugh Hampton Young



Annual Meeting, American Urological Association, Inc., New Orleans, Louisiana, May 13-17, 1990

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51061-283

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LOCAL AND SYSTEMIC EFFECTS OF CHRONIC INTRACAVERNOUS INJECTION OF PAPAVERINE, PROSTAGLANDIN E₁, AND SALINE IN PRIMATES

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ABSTRACT

To compare the local and systemic effects of chronic intracavernous injection of papaverine, prostaglandin E₁, and saline on erectile tissue, eight pigtail monkeys underwent 75 injections over a nine-month period. Monkeys were divided into three groups; each group received papaverine (10 mg.), prostaglandin E₁ (20 µg.), or saline (one ml.). The erectile response was closely observed for two hours after each injection to monitor the onset, degree, and duration of erection. Liver function tests were performed every three months to detect early systemic metabolic changes. After sacrifice, the simian penises were perfused in situ and examined histologically with both light and electron microscopy.

Papaverine resulted in an initially strong erectile response, but this was maintained throughout the length of the study in only two monkeys. In contrast, prostaglandin E₁ resulted in tumescence that was maintained in all monkeys over the nine-month period. In addition, the papaverine group had elevated liver enzymes and significant histologic changes with loss of normal architecture on both light and electron microscopy. The other two groups showed only minimal histologic changes or none. (*J. Urol.*, 142: 403-408, 1989)

During the past four years, the intracavernous injection of various vasoactive agents has attracted the attention of many investigators and opened a new avenue for both the diagnosis and management of erectile dysfunction.

The ability of vasoactive substances to induce erection was first reported in 1982 by Virag,¹ when the accidental intracavernous injection of papaverine during a shunting procedure produced erection. Since then, several clinical and laboratory studies have reported the same effect with intracavernous injection of other substances, including phenoxybenzamine,² vasoactive intestinal polypeptide,³⁻⁶ and a calcium channel blocker (Wagner, G.: personal communication). However, at this time papaverine, either alone or in combination with the alpha-1 blocker phentolamine,^{7,8} has assumed primary importance and has been successfully used not only for treatment but also for the diagnosis of erectile dysfunction.⁹⁻¹¹

Despite the effectiveness of these agents, serious local and systemic side effects have been reported: priapism,^{11,12} fibrosis,¹³ dizziness, hypotension, and even one death.¹⁴

Prostaglandin E₁ (PGE₁) is a potent smooth-muscle relaxant that occurs naturally in body fluids and has been found in high concentrations in seminal fluid.¹⁵ Hedlund and Andersson¹⁶ have reported its ability to induce relaxation of isolated human penile erectile tissue and cavernous arteries. Moreover, intracavernous injection of PGE₁ has been proven safe and effective in both European^{17,18} and Japanese¹⁹ clinical trials. The fact that PGE₁ is probably metabolized by penile tissue and is less acidic than papaverine (pH = 5.0 vs. 3.1) would at least theoretically suggest that it would result in fewer complications than those commonly seen with papaverine and alpha-1 blockers.

To identify the most effective substance with the fewest side effects, we undertook this study to compare the effects of

repeated intracavernous injection of papaverine, PGE₁ and saline on erectile tissue in monkeys.

MATERIALS AND METHODS

Eight adult pigtail monkeys, weighing 4.8 to 5.2 kg., were divided into three groups for intracavernous injections: Group 1 (three monkeys) received 10 mg. papaverine (papaverine hydrochloride; Lilly); Group 2 (three monkeys) received 20 µg. PGE₁ (Prostin VR Pediatric; Upjohn); and Group 3 (two monkeys) received normal physiologic saline (one ml.). All monkeys were given a total of 75 injections.

For each injection, the animals were sedated with ketamine hydrochloride (40 mg./kg. body weight) intramuscularly, repeated when necessary to ensure adequate sedation with spontaneous respiration. A rubber band was placed tightly around the base of the penis and the penile skin was properly cleansed with an antiseptic solution. The agent was injected with a 27-gauge needle on the lateral aspect of the mid-portion of the slightly stretched penis proximal to the attachment of the foreskin to avoid subcutaneous hematoma. All injections were limited to one side only. After the drug was delivered, the needle was removed and the puncture site was compressed manually between two fingers. Manual compression and rubberband constriction were maintained for one minute to ensure adequate distribution of the drug into the cavernous tissue and prevent hematoma formation.

The erectile response was closely observed for two hours to monitor the onset, degree and duration of tumescence. To elucidate the erectile response, a subjective grading scale was used: full erection; tumescence; weak response; and no response (full erection = penile angle of 90°, with rigidity; tumescence = maximal increase in length and girth, but no rigidity; weak response = slight increase in length and girth only).

Injections were given twice a week until a total of 75 injections was reached. Liver function tests (SGOT, SGPT) were performed at the beginning of the study and every three months thereafter to detect early systemic metabolic changes.

Accepted for publication February 6, 1989.

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All animals were sacrificed after 75 injections. The penises were perfused in situ with Bolar's fixative (three per cent glutaraldehyde, two per cent paraformaldehyde in sodium cacodylate buffer) and the cavernous tissue was stained with hematoxylin and eosin and trichrome stain for light microscopy and uranyl acetate-lead citrate for electron microscopy.

For better localization of pathologic changes, the penises were divided into three segments: proximal to the site of injection; the site of the injection; and distal to the site of injection. Each segment was further subdivided into three sections, each about 0.5 cm. thick. As a control, penile tissue samples from two monkeys of the same species that had not undergone injection were prepared in the same fashion for histologic studies.

RESULTS

Erectile response to intracavernous injection. Both papaverine and PGE₁ resulted in a good erectile response initially. However, with repeated injections the responses and complications varied between the two groups (table 1).

Papaverine group. At the beginning of the study, the three monkeys in this group showed an excellent erectile response, full erection that lasted for 30 to 40 min. None of the animals developed priapism with this 10-mg. dose. The strength and the duration of the response were maintained for about 30 injections in two monkeys and for only 22 injections in the third. In the first two, although the degree of erection remained the same, the duration decreased slightly to 20 to 25 min. until injection no. 67 in the first and no. 52 in the second, when it decreased to 10 min. in both and continued of this duration until the end of the study. Slight penile curvature and induration were noted, as was difficulty with injection. In the third monkey, cavernositis developed after 22 injections, but resolved with antibiotic therapy for one week. The animal continued to respond to injection, but the strength and duration of the erectile response gradually decreased until it was completely lost after 52 injections. Towards the end of the study, the penis appeared retracted and fibrotic, and there was marked difficulty in skin puncture and drug delivery.

Prostaglandin E₁ group. Intracavernous injection of PGE₁ resulted in tumescence in all animals, which lasted for about 10 to 15 min. This response was essentially maintained throughout the study. One monkey in this group (No. 5) died of anaphylactic shock in response to ketamine after 61 injections. None of the animals showed any clinical evidence of fibrosis.

Saline group. The intracavernous injection of physiologic saline elicited no erectile response throughout the study.

Liver function studies. Two monkeys in the papaverine group showed an elevation in SGPT (152 IU in one, 98 IU in the second; normal = 35–55 IU) after about 60 injections, which persisted until the end of the study. The other six monkeys

showed no changes. Liver biopsy in these two animals, as well as in the others, showed no significant pathologic changes.

Histologic examination: light microscopy. The monkey penis, similar to the human penis, consists of two corpora cavernosa separated by an incomplete septum, which is deficient distally. Each cavernous body consists of numerous endothelium-lined sinusoidal spaces separated by connective tissue trabeculae containing bundles of smooth muscle cells, nerve fibers and arterioles (fig. 1).

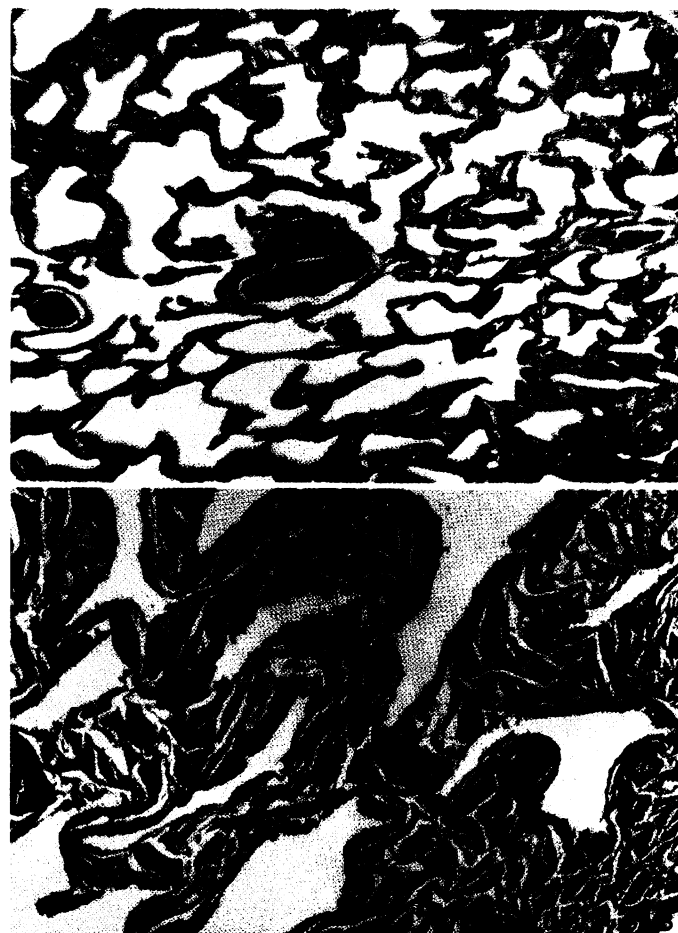


FIG. 1. A, under light microscopy, complete section of cavernous tissue from control specimen shows endothelium-lined sinusoidal spaces separated by connective tissue trabeculae (trichrome stain, magnification $\times 20$). B, control specimen under greater magnification ($\times 85$).

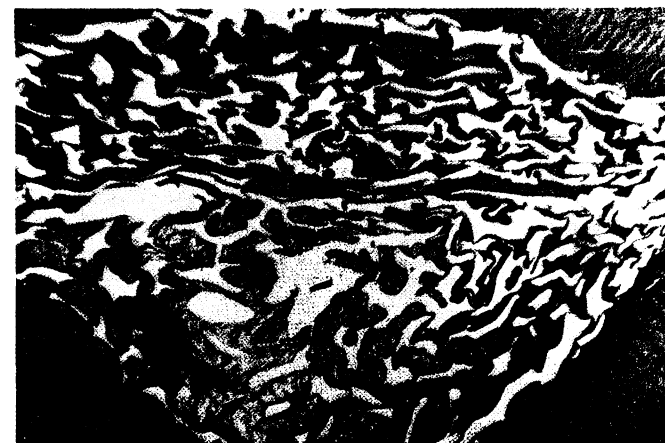


FIG. 2. Cavernous tissue from saline-treated group shows localized area of fibrosis at site of injection (trichrome stain, $\times 20$).

TABLE 1. Erectile response to 75 intracavernous injections of papaverine, PGE₁ and saline in monkeys

Group	Erectile Response			
	Full Erection	Tumescence	Weak Response	No Response
No. 1	60	13	2	0
No. 2	64	8	3	0
No. 3	24	4	24	23
Prostaglandin E ₁				
No. 4	3	68	0	4
No. 5	6	51	0	4
No. 6	4	69	0	2
Saline				
No. 7	—	—	—	75
No. 8	—	—	—	75

In the saline-treated group, the architecture of the cavernous tissue in the proximal and distal segments was well preserved and appeared similar to the control tissue samples. However, in two sections in the middle segment (at the site of the injection), there was a localized area of mild fibrosis and degeneration of the smooth muscles (fig. 2).

Samples from all three monkeys in the papaverine group showed significant histologic changes with loss of normal architecture, both at the site of the injection and in the proximal and distal segments. A variety of pathologic changes were seen:

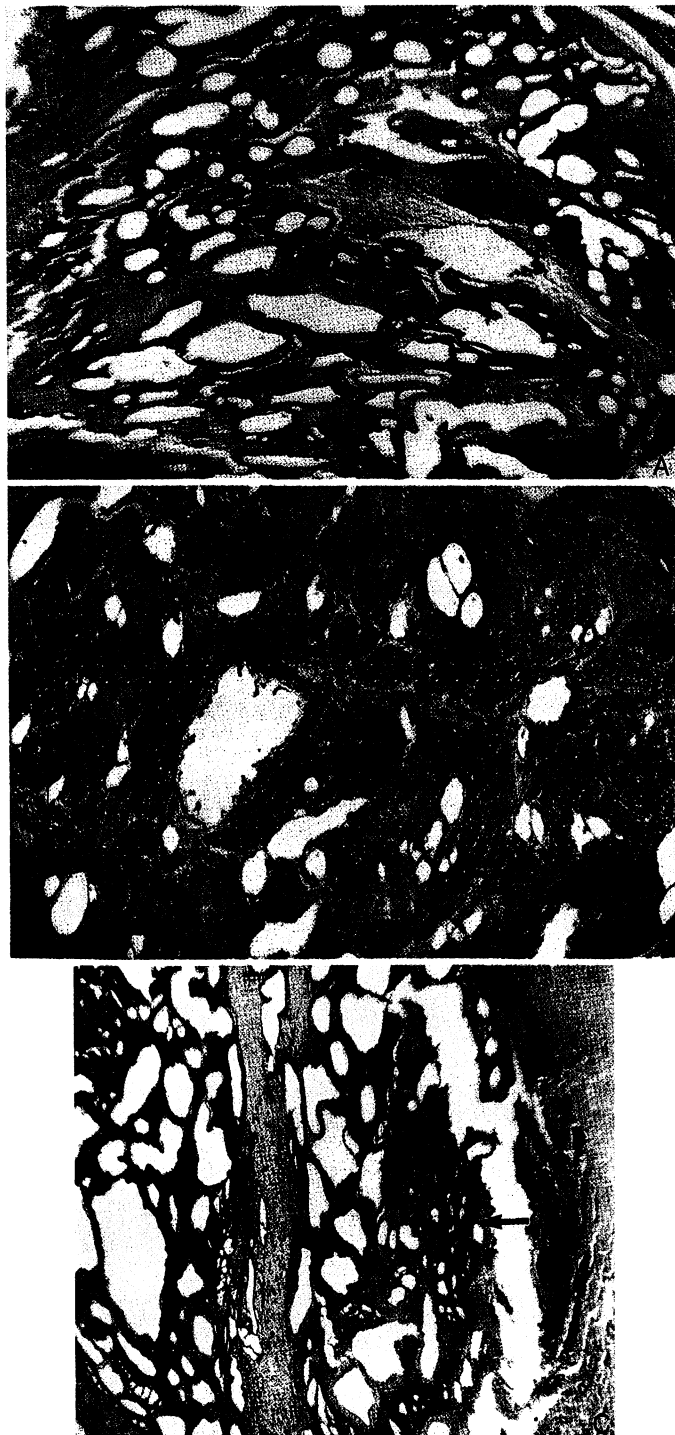


FIG. 3. Cavernous tissue from papaverine group shows: A, significant fibrosis with complete loss of normal architecture (trichrome stain $\times 25$) and B, marked smooth muscle hypertrophy ($\times 85$). C, in section of corpora separated by septum, smooth muscle hypertrophy is demonstrable on one side and fibrosis on other ($\times 20$).

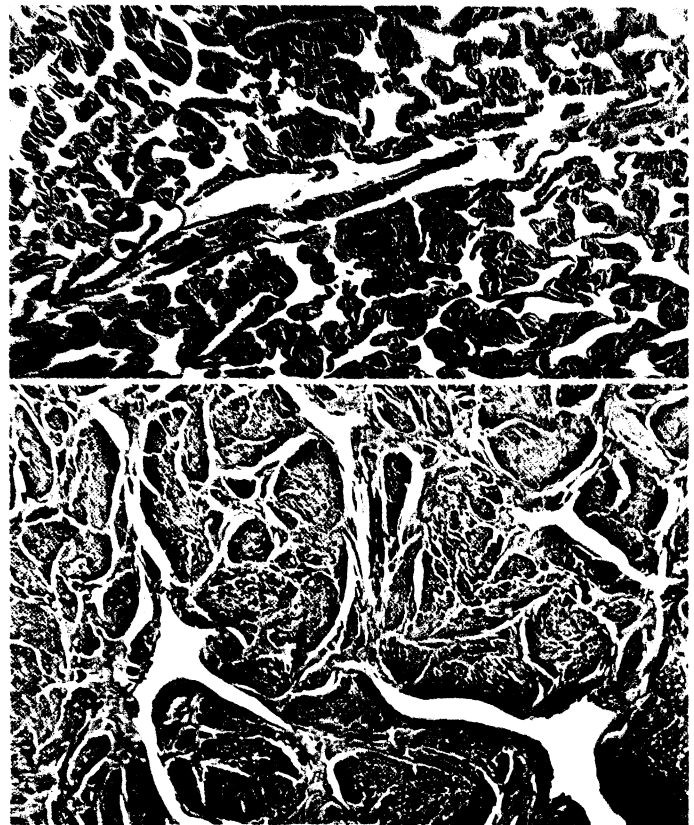


FIG. 4. A, cavernous tissue from PGE_1 group shows only slight degree of smooth muscle hypertrophy with otherwise normal architecture ($\times 16$). B, similar specimen under greater magnification ($\times 85$).

mild to severe fibrosis with an increase in the amount of collagenous tissue, edema, degeneration and atrophy of the smooth muscles (fig. 3A); varying degrees of thickening and hypertrophy of the smooth muscle (fig. 3B). Interestingly, in the segments where both corpora are separated by the septum, there was more pronounced fibrosis with fragmentation and atrophy of the smooth muscles on the injected side, whereas the other side showed prominent smooth-muscle hypertrophy with a relative decrease in the amount of intervening loose areolar tissue and consequently a relative decrease in the size of the sinusoidal spaces (fig. 3C).

Few pathologic changes were found in the PGE_1 group (fig. 4A). Similar to the findings in the saline group, a very localized area of increased collagen and a decrease in smooth muscle mass were seen in a few sections from the middle segment. In addition, a slight degree of smooth-muscle hypertrophy, less than that seen in the papaverine group, was consistently seen in the proximal and distal segments in all three monkeys (fig. 4B).

Transmission electron microscopy (TEM). This confirmed the findings seen under light microscopy. Specimens from the two control monkeys and from the saline group showed smooth muscle bundles with loose areolar tissue, nerve bundles and arterioles in the trabeculae (fig. 5). In the PGE_1 group, the normal architecture was preserved; however, the smooth muscles showed a slight degree of hypertrophy compared with the control animals and the saline group (fig. 6). In the papaverine group, TEM showed complete distortion of the normal histologic findings. In some areas, the smooth muscles showed a severe degree of hypertrophy with infolding of the plasma membrane and a marked reduction in the amount of intervening loose areolar tissue (fig. 7A). In other areas, especially on the injected side, atrophy and degeneration of the smooth muscles with replacement of the whole area by collagenous tissue were the dominating feature (fig. 7B).

DISCUSSION

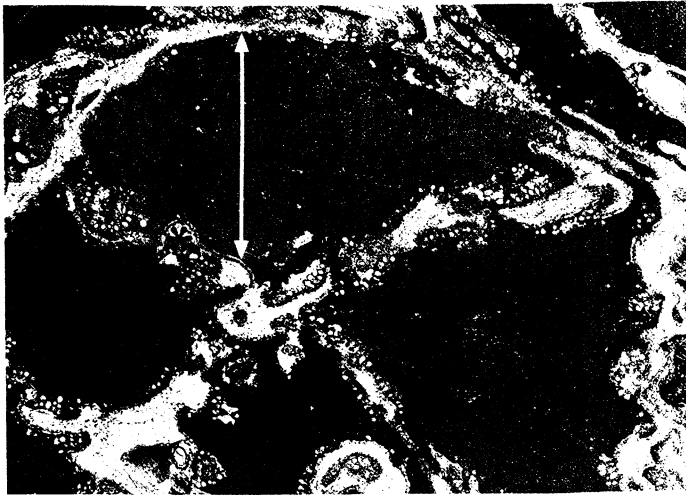


FIG. 5. Transmission electron microscopy of control specimen shows bundles of smooth muscles with intervening connective tissue (*) ($\times 9000$). Compare with hypertrophied smooth muscle in fig. 7A.

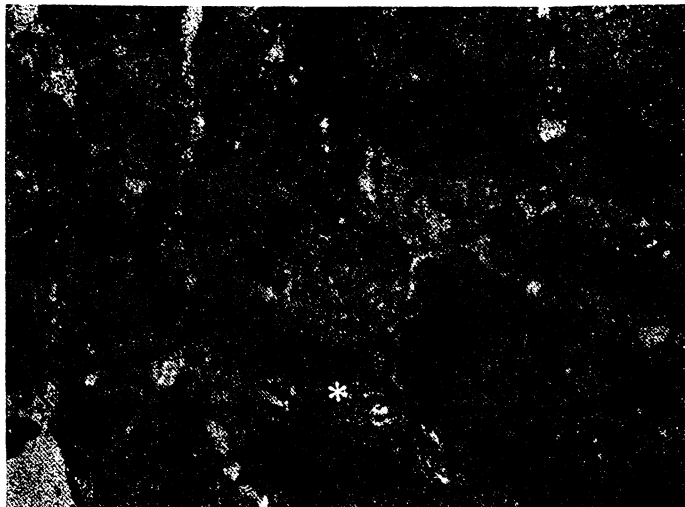


FIG. 6. TEM from PGE₁ group shows slight increase in size of smooth muscles with relative decrease in connective tissue trabeculae (*) ($\times 9000$).

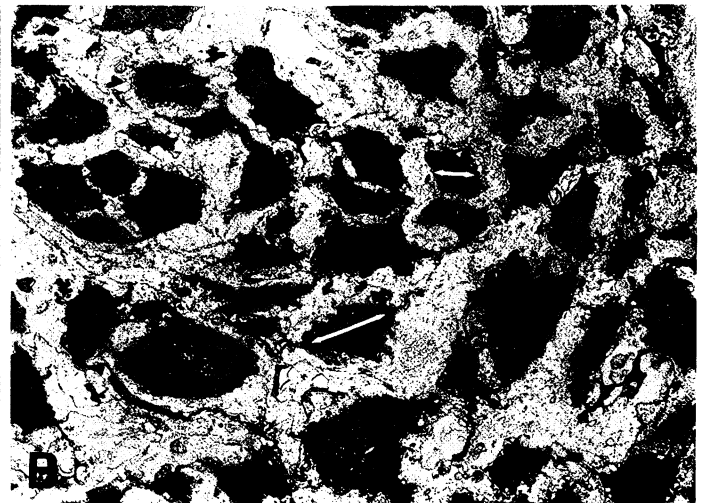
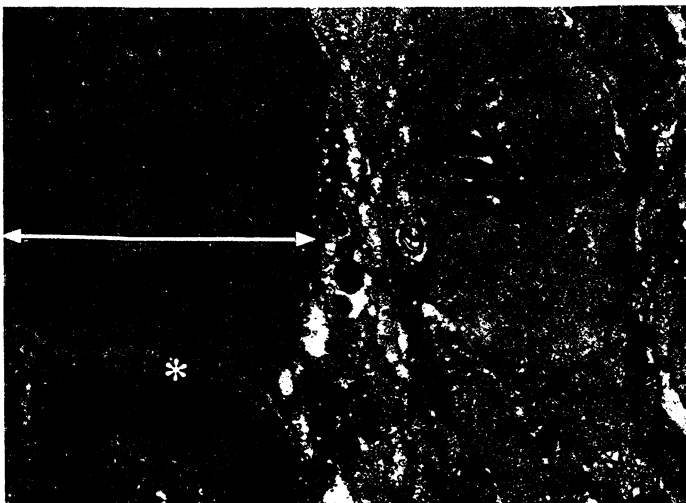


FIG. 7. TEM from papaverine group shows: A, marked increase in size of smooth muscles (compare with fig. 5) ($\times 9000$), and B, atrophy and degeneration of smooth muscles ($\times 3000$).

Pharmacologic treatment of impotence has gained widespread acceptance. Indeed, the use of the intracavernous injection technique has increased rapidly, although the ideal agent has yet to be identified. We believe that the ideal drug for intracavernous injection is that which results in a good erectile response of reasonable duration and which can be used repeatedly with minimal local or systemic side-effects or none.

For a vasoactive agent to be effective in producing erection, it should increase arterial flow, sinusoidal relaxation, and venous resistance.²⁰⁻²³ Papaverine, a potent smooth-muscle relaxant whose effect derives from either inhibition of cyclic adenosine monophosphate/phosphodiesterase²⁴ or an antinicotinic action,²⁵ has been shown to do so in animal^{26,27} and human studies.^{10,11}

Similarly, PGE₁, which has been reported to diminish the output of norepinephrine,²⁸ consequently blocks the adrenergic tone in cavernous smooth muscle. Stackl et al.²⁹ have also shown that PGE₁ increases arterial blood flow in the cavernous artery in humans. In addition, human clinical trials of intracavernous injection of PGE₁ have shown its effectiveness, even in patients who don't respond to other agents, with no systemic or local side effects.^{12,17,18} The action of PGE₁ on norepinephrine output might be of value, especially in patients with psychogenic impotence.

It appears from both animal and clinical studies that both papaverine and PGE₁ are capable of producing erection by bypassing the neurogenic influence through those two different mechanisms.

In our study, papaverine resulted initially in full erection, whereas PGE₁ caused only tumescence. We believe that this is probably due to the fact that papaverine exerts its action nonspecifically on the cavernous tissue, whereas PGE₁ interacts with specific receptors on the effector cells, which vary in number with different species.³⁰ Roy et al.³¹ and Saenz de Tejada et al.³² have reported that human cavernous tissue can generate prostaglandins *in vitro*. We are currently performing more studies to document and quantify the number of PGE₁ receptors in the corpora cavernosa of both men and primates.

Despite the initial effectiveness of both drugs, the pattern of erectile response varied considerably with repeated injections. The PGE₁ group maintained their erectile response with essential consistency and without any complications or clinical evidence of fibrosis. In contrast, the papaverine group showed some loss of erectile capability over time, evidenced by marked

shortening in the duration of erection (two monkeys) and complete loss of response (one monkey). The gradual decrease of the erectile response was explained by the histologic findings, which showed marked degeneration and atrophy of the smooth muscles and their replacement by collagen fibers, resulting in a change in the compliance of the sinusoidal system.

In addition to the clinical evidence of local induration and fibrosis of the penis in the papaverine group, two monkeys showed a significant elevation of liver enzymes without any pathologic changes in hepatic architecture. We are not sure whether this represents a systemic side effect of chronic papaverine use or resulted from the ketamine anesthesia. However, the absence of elevated liver enzymes in the other animals and the previous reports of papaverine-induced chronic liver disease³³⁻³⁵ make the first possibility more likely. We feel that this systemic side effect requires further investigation and warrants caution.

Similar to the discrepancy in the erectile response, results of histologic examination of the cavernous tissue varied markedly among the three groups and revealed interesting information. The consistent finding of a localized area of mild fibrosis at the site of repeated needle puncture would signify that intracavernous injection therapy is traumatic, regardless of the agent used. Recent reports of an implantable intracavernous drug delivery system³⁶ as well as with the transcutaneous route³⁷ may offer an alternative. Both PGE₁ and papaverine showed smooth-muscle hypertrophy, which was more pronounced in the latter group. This makes us believe that repeated injection of any vasoactive agent will cause smooth-muscle hypertrophy whose degree is probably relative to the drug's duration of action. The fact that PGE₁ is metabolized locally within the cavernous tissue³⁸ may explain the absence of prolonged erections commonly seen with papaverine injections³⁹ and the consequently lesser degree of smooth-muscle hypertrophy.

In addition to the marked degree of smooth-muscle hypertrophy and loss of connective tissue trabeculae in the papaverine group, there was severe fibrosis, degeneration and atrophy of the smooth muscles with a complete loss of normal architecture. These findings agree with previous studies in animals¹³ and with clinical observations in humans,^{7,40} where fibrosis and angulation of the tunica albuginea have been reported after repeated injection. One can hypothesize from the absence of severe fibrosis in the other two groups that the local irritation of the cavernous tissue from papaverine may result from its high acidity.⁴¹ The facts that the pH of PGE₁ is similar to that of saline (5.0) and that it is an endogenous substance that is locally metabolized make it less irritating to the cavernous tissue.

From our study, one can conclude the following: Intracavernous injection of both papaverine and prostaglandin E₁ produces a good erectile response; however, the decrease in response and the marked histologic changes and complete loss of normal architecture over time with chronic papaverine use make PGE₁ the more appropriate agent. In addition, the ability of PGE₁ to diminish the output of norepinephrine may render it more efficient in the differential diagnosis of psychogenic impotence.

The intracavernous injection technique is an excellent mode of therapy in patients with erectile dysfunction. However, it is not absolutely safe, as the unavoidable trauma from repeated needle puncture results in an area of fibrosis at the site of injection, regardless of the agent used. In addition, repeated injection of any vasoactive agent may result in some degree of smooth-muscle hypertrophy.

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